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Acceleration of formaldehyde reactions with proteins due to ultrasound

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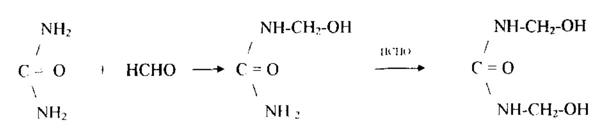
Abstract

We have found that the reactions between caseine and formaldehyde or Urotropin are markedly increased under the influence of ultrasound. We also present a probable interpretation of the mechanism causing the acceleration of these reactions.

Keywords: Condensation; Formaldehyde; Protein; Ultrasound; Urotropin

1. Introduction

Formaldehyde reacts with amino groups, which is well known because in an alkaline environment with urea it forms monomethylolurea and dimethylolurea while continued reaction ends in the production of resins of melamine and formaldehyde [1].



Using the same method, the hexamethylentetramine is prepared with the intermediate production of trimethylolamine the hydroxyls of which react with the hydrogen atoms of ammonia [2]. Urotropin in an acidic environment breaks up, producing formaldehyde which has disinfectant properties in the urinary system [3]. Formaldehyde also reacts with the imino groups of albumins resulting in the plasticisation of the latter (caseine plastics) [4]. The embalming of animals is based on this reaction together with the disinfectant properties of the formaldehyde. In our experiments, we found that ultrasound of an intensity above the cavitation threshold accelerated reactions between albumins and formaldehyde (or Urotropin).

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2. Experimental

The transducer had a diameter of 2.54 cm, a frequency of 0.8 MHz and the average intensity of the produced beam was 4 W/cm^2. The samples used in our initial experiments were of caseine, cylindrical in shape, 2 cm high and 2 cm in diameter, made from the coagulation of ordinary skimmed milk with hydrochloric acid. The samples were mixed with a solution of 35% content of formaldehyde in water (Formol), wrapped in polyethylene film and immersed in water. This was done for the following reasons: (a) to secure the propagation of the ultrasound to the samples through the water; and (b) to maintain the temperature of the samples as constant as possible. After sonication of the samples for 30 min, the hardness of the surfaces on the side exposed to the transducer was measured. The measurements were performed with a penetrometer having a needle with a 1 mm^2 cross-section. The results relating to the hardness of the surfaces are given in p/mm^2 in Table 1.

Sample 4 was measured without ultrasound continuously at regular time intervals until its hardness reached the level of 40, which is the hardness of sample 3 (sonicated for 30 min). This hardness was reached after 13 h, indicating the benefit of ultrasound.

A coagulum of caseine was prepared (Sigma Chemicals C-3400 from bovine milk) in the following manner: in 1000 ml of water 4 g of sodium hydroxide and 60 g of caseine were dissolved. One hour later 12 ml of concentrated hydrochloric acid was added to precipitate the caseine.

Table 1
Ultrasonic radiation results on caseine (impure) and formaldehyde mixed together

Sample	Formalin (cm^3)	Urotropin solution (cm^3)	Ultrasonic radiation (min)	Hardness (p/mm^2)
1	—	—	—	2.3
2	—	—	30	6.8
3	0.5	—	30	40.0
4	0.5	—	—	8.0
5	—	0.5	30	11.0
6	—	0.5	—	2.8

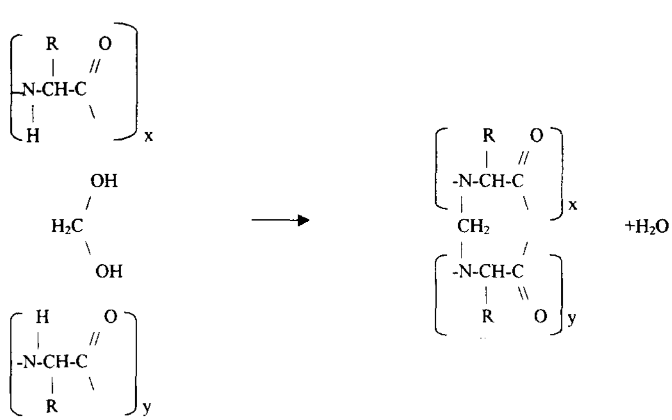
Table 2
Results as in Table 1 but with pure caseine

Sample	Formol	Ultrasonic radiation (min)	Hardness (p/mm^2)	
			After 30 min	After 24 h
1	—	—	40	50
2	—	30	130	160
3	Yes	30	340	570
4	Yes	—	60	140

The redundant water was eliminated and the water in the solid absorbed with filter paper. As a result, caseine was obtained with 75% humidity and with a total weight of 240 g. Four samples were prepared from the pulp, each of 17.5 g. Samples 1 and 2 of caseine pulp were mixed with an additional 0.5 ml of water; samples 3 and 4 were mixed with 0.5 g Formol (35% formaldehyde). The resulting samples were formed into cylinders (without compression) to a diameter of 2 cm and height of 1 cm, disregarding the surplus. Sample 1 was used as a control. The samples were then wrapped in polyethylene film and immersed in water 19°C. The experiments were carried out as described at the beginning of this section and the results are shown in Table 2.

3. Conclusions

As a first observation, the casein we prepared from skimmed milk (Table 1) was not as chemically pure, and so not as hard as that supplied by Sigma Chemicals (Table 2). This is due to the fact that the milk used contained other organic substances such as lactose (milk-sugar) etc. However, both sets of experiments show a high (very intense) acceleration of the chemical reaction of casein with formaldehyde when exposed to ultrasound. The results are much more positive when pure casein was used, and this can be seen by comparing samples 3 and 4 of the two tables. Indeed, sample 4 in Table 1, without ultrasound, reached a hardness value



Scheme 1.

of 40 p/mm^2 after 13 h. By comparison, sample 4 of Table 2 did not reach the hardness of sample 3 (340 p/mm^2) in 30 min with ultrasound, even after 24 h.

Formaldehyde was replaced with Urotropin in some experiments because Urotropin, in small doses, is not toxic to living organisms. The results obtained are very encouraging, offering the possibility of tumour treatment using Utropin. The increase in hardness with time, for all the samples, must be at least partially attributed to the of removal of water.

A probable interpretation of these results is that ultrasound breaks up the water through cavitation [5] creating free radicals, which, with the formaldehyde create an intermediate alcohol as follows:

$$H_2O \xrightarrow{U.S} \dot{H} + \dot{O}H \xrightarrow{H_2CO} H_2C(OH)_2$$

This alcohol reacts with the albumins, combining them with the nitrogen of the imino groups in the peptide bond as shown in Scheme 1.

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